Citation:

Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Fukuda K, Muto Y, Kondo K. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *Am J Clin Nutr*. 2007 Mar;85(3):709-17.

PubMed ID: 17344491

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

This study tested whether long-term intake of cocoa powder alters plasma lipid profiles in normocholesterolemic and mildly hypercholesterolemic human subjects.

Inclusion Criteria:

- Healthy males
- Normal body weight
- Nonsmokers
- No evidence of chronic disease

Exclusion Criteria:

- Consuming more than 25 mL alcohol/day
- Taking medications, antioxidants, or vitamin supplements

Description of Study Protocol:

Recruitment - Methods not described

Design - Randomized controlled trial

Blinding used (if applicable) double blind

Intervention (if applicable)

Subjects were divided into 2 groups according to BMI, and plasma total, LDL, and HDL cholesterol concentrations and were then instructed to consume one of the following test drinks

daily for 12 weeks:

- 12g sugar/day (control group)
- Mixture of 26g cocoa powder and 12 g sugar/day (cocoa group)

Cocoa powder was consumed as a beverage after the addition of hot water, the the test drinks being consumed twice each day: before noon and during the afternoon.

Home-delivered food was provided to each subject to ensure the same foods were consumed in the 3 days before collection of blood and urine samples. Subjects were asked to maintain their normal diets and avoid all other cacao products while leading their usual lifestyle during the 12 weeks.

Statistical Analysis

- Data expressed as means+/- SEMs
- Change from baseline (12 wk - baseline) in the control and cocoa groups were compared by using repeated-measures ANOVA and unpaired t tests to assess whether a significant group x time interaction had occurred
- Mixed model analysis was used to examine the interaction between 2 risk factors with time and the risk factors acting as the independent variables
- If a significant interaction was found, separate correlations were calculated at baseline and 12 wk using Pearson's correlation analysis. P value < 0.05 significant.

Data Collection Summary:

Timing of Measurements

- At baseline and 12 weeks:
  - subjects fasted for 12 hours, and then blood samples were collected and 24-hour urine samples were collected from 0900 the day before the blood collection until 0900 of the day of the collection
  - Body weight, blood pressure (BP), and heart rate (HR) were measured
- Subjects kept complete dietary food records throughout the study
- Safety measurements were collected at baseline and 12 wk: plasma total protein, albumin, glucose, uric acid, urea nitrogen, creatinine, free fatty acids, phospholipids, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase, alkaline phosphatase, lactate dehydrogenase, sodium, potassium, chloride, and calcium; urine samples were used to monitor proteinuria, glucosuria, urobilinogen, and occult blood.

Dependent Variables

- Plasma LDL oxidative susceptibility - fasting blood draw; measured as the lag time of conjugated diene production formed by a radical generator (expressed in minutes)
- Plasma lipids and oxidative LDL - fasting blood draw; plasma VLDL, LDL, and HDL cholesterol concentrations were measured by a rapid electrophoresis scanning automated system with the use of agarose-gel electrophoresis; triacylglycerols (TG) was assayed using standard technique; oxidized LDL in plasma was measured using monoclonal antibody mAb-4E6
- Urinary oxidative stress markers - 24-hour urine; measured via liquid chromatography
- Urinary catechin and epicatechin - total amount excreted in 24-hour urine; analyzed by LC-MS

Independent Variables

- Cocoa group (control or cocoa)
- Food records - 3 day food records were analyzed with the Excel Food Frequency Questionnaire (Japan) on days 1-3, 26-28, 54-56, and 80-82.

Control Variables

Description of Actual Data Sample:

Initial N: 25 males

Attrition (final N): 25 males

Age: 38 ± 1 year

Ethnicity: Not reported

Other relevant demographics: Not reported

Anthropometrics

- Mean body weight: 64+/- 1 kg
- Mean body mass index 22.1+/0.2 kg/m^2
- Plasma total cholesterol: 4.65-6.41 mmol/L
- Plasma LDL cholesterol: 2.46-4.92 mmol/L
- Plasma HDL cholesterol: 0.75-2.60 mmol/L

Location: Japan

Summary of Results:

Key Findings

- The prolongation from baseline levels in the lag time of LDL oxidation in the cocoa group (9%) was significantly greater than the reduction measured in the control group (-13%).
- The HDL cholesterol in the cocoa group (24%) increased significantly more than the control group (5%).
- Negative correlation between plasma concentrations of HDL cholesterol and oxidized LDL.
- At 12 weeks, there was a 24% reduction in dityrosine from baseline concentrations in the cocoa group, which was significantly greater than the reduction in the control group (-1%).

Variables	Control Group Measures and confidence intervals	Cocoa group Measures and confidence intervals	Statistical Significance of Group Difference
BMI (kg/m^2)	Baseline: 22.1+/-0.3	Baseline: 22.1+/-0.4	NS
	12 weeks: 21.5 +/-0.3	12 weeks: 21.6 +/-0.4	NS
Systolic BP (mmHg)	Baseline: 117 +/- 2	Baseline: 124 +/- 3	NS
	12 weeks: 120 +/- 3	12 weeks: 122 +/- 2	NS

Diastolic BP (mmHg)	Baseline: 79 +/- 2	Baseline: 77 +/- 2	NS
	12 weeks: 77 +/2	12 weeks: 75 +/- 2	NS
HR (beats/min)	Baseline: 71 +/- 4	Baseline: 77 +/-2	NS
	12 weeks: 72 +/- 3	12 weeks: 77 +/- 3	NS
Lag time (minutes)	Baseline: 58.6+/-3.8	Baseline: 57.4+/-3.1	Change (wk 12-baseline) -11.6+/-3.8 for control and 5.4+/-2.9 for cocoa; p<.001
	12 weeks: 47.0+/-2.3	12 weeks: 62.8+/-1.8	
HDL cholesterol (mmol/L)	Baseline: 1.36+/-0.15	Baseline: 1.37+/-0.11	Change 0.08+/-0.03 for control and 0.31+/-0.05 for cocoa; p<.001
	12 weeks: 1.43+/-0.15	12 weeks: 1.69+/-0.13	
Dityrosine (micromol/24h)	Baseline: 74.1+/-6.2	Baseline: 91.7+/-8.1	Change -0.8+/-5.2 for control and -21.6+/-8.5 for cocoa; p<.05
	12 weeks: 73.3+/-5.4	12 weeks: 70.1+/-7.6	

Other Findings

- Baseline values of plasma biochemical variables, lipids, oxidized LDL concentrations, LDL susceptibility, and urinary oxidative stress markers did not differ significantly between groups.
- No subjects reported adverse events resulting from cocoa intake
- No significant differences between mean energy and nutrient intake (protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrate, cholesterol, or vitamins C and E) were observed between the groups during the 3-day periods that dietary records were analyzed.
- No significant differences in the change from baseline to 12 weeks for plasma total cholesterol, VLDL, or LDL cholesterol or triacylglycerol.
- The 2 groups did not differ in their change in urinary stress markers from baseline to 12 weeks, except for dityrosine (see table above).
- After 12 weeks, there was an 8-fold increase in catechin excretion and a 10-fold increase in epicatechin excretion in the cocoa group. These increases resulted in the urinary excretion of both catechin and epicatechin being significantly higher at 12 wk. in the cocoa group than in the control group (P<.001 for both).
- There were no differences in safety measurements between groups.

Author Conclusion:

Consumption of cocoa powder containing polyphenolic substances at a dosage of 26 g/day for 12 weeks increased the resistance of LDL to oxidation and raised HDL-cholesterol concentrations in the plasma in normocholesterolemic and mildly hypercholesterolemic humans. Increases in

HDL-cholesterol concentrations may contribute to the suppression of LDL oxidation.

Reviewer Comments:

Strengths:

- *RCT provides strong research design*
- *Dietary food records analyzed for 3-day periods throughout the study*

Weaknesses:

- *Only male subjects limits the generalizability to women*
- *Small sample size*
- *The cocoa group started with a significantly higher dityrosine than the control group, which may be a factor in the significant change from baseline to 12 weeks in the cocoa group (the control group was virtually unchanged).*
- *No cross-over in the design*
- *Physical activity not tracked during the study (as a control variable)*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	No

2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	No
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	???
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	???
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	No
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	No

	7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**		Yes
	8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		No
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**		No
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	No